**Fat provisioning in winter impairs egg production during the following spring: a landscape-scale study of blue tits**

# Fat provisioning in winter impairs egg production during the following spring: a landscape-scale study of blue tits

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**Running title:** Winter provisioning effects on egg production

## SUMMARY

**1.** Provisioning of garden birds is a growing phenomenon, particularly during winter, but there is little empirical evidence of its true ecological impacts. One possibility is that winter provisioning could enhance subsequent breeding performance, but this seems likely to depend on the types of nutrients provided. For example, whereas effects of macronutrients such as fat are unlikely to be carried over to influence breeding in small passerines, micronutrients such as dietary vitamin E (an antioxidant) may be stored or have lasting health benefits.

**2.** Here, we examine the carry-over effects of winter food supplements on egg production in wild populations of blue tits (*Cyanistes caeruleus*). Over three consecutive years birds were provisioned with fat, fat-plus-vitamin E, or remained unfed (controls).

**3.** The provision of fat in winter resulted in smaller relative yolk mass in larger eggs, and reduced yolk carotenoid concentrations in early breeders. However these effects were not seen in birds provisioned with fat-plus-vitamin E. Lay date, clutch size, egg mass and yolk vitamin E concentrations were not significantly affected by winter provisioning treatment.

**4.** Our results indicate that winter provisioning can have important downstream consequences, in particular affecting investment in egg production several weeks or months later.

**5.** Provisioning is widely applied to support garden bird populations, and for the conservation management of endangered species. However, our results challenge the assumption that such practices are always beneficial at the population level, and emphasise how the ecological impacts can depend on the specific nutritional profile of provisioned foods.



**Key words:** Antioxidant; carry-over effect; egg quality; life-history trade-off; maternal effect; supplementary feeding; vitamin E

## INTRODUCTION

The evolution of life-history traits is constrained by the existence of trade-offs amongst them; at the proximate level such trade-offs are often modulated by variation in the supply of dietary resources (Stearns 1992). Indeed, food supply plays a crucial role in avian ecology, by influencing community structure, regulating population sizes and through effects on individual behaviours and life-histories (Newton 1998). For birds in temperate climates, access to sufficient dietary resources is likely to vary seasonally, leading Lack (1954) to hypothesise that populations would be regulated by food availability during the period of least abundance; the 'winter food limitation hypothesis'. Consequently, over-winter food supply has been shown to enhance body condition and survival in the short-term (Jansson, Ekman & Vonbromssen 1981; Brittingham & Temple 1988; Grubb & Cimprich 1990). This is of particular relevance to the *ad hoc* supplementation of foods within urban habitats, a hugely popular and growing phenomenon worldwide (Jones & Reynolds 2008). Provisioning of garden birds is most prevalent in winter months, when energetic demands are high and natural food is relatively scarce (Chamberlain *et al.* 2005). Indeed, the UK and US together purchase in excess of 500,000 tonnes of commercial bird food each year (O'Leary & Jones 2006), but the ecological consequences of this enormous resource are little studied (Jones & Reynolds 2008; Robb *et al.* 2008a).

Whilst the capacity for winter food availability to affect survival is well established, there can be other, downstream consequences that remain poorly understood. Carry-over effects arise when events in one season or year influence an individual's performance in a later season or year (Harrison *et al.* 2011). The potential for natural food availability on the wintering grounds to influence subsequent productivity in migratory birds is well known (reviewed by Harrison *et al.* 2011). However while numerous experimental studies have investigated the

effects of food supplementation just prior to or during breeding on productivity in wild birds (e.g. Svensson & Nilsson 1995; Blount *et al.* 2002b), effects across seasons have rarely been quantified (Park, Lee & Rhim 2004; Robb *et al.* 2008b). In a landscape-scale investigation, Robb *et al*. (2008b) found that blue tits provisioned with peanuts during winter had advanced laying dates and increased fledging success compared to unfed controls, even though provisioning had stopped 6 weeks prior to breeding.

The mechanisms that underlie such carry-over effects of winter provisioning on garden birds are poorly understood. Until recently, it has been assumed that carry-over effects are largely driven by energy availability. However, as 'income breeders', small passerines cannot store macronutrients such as fat to any great extent and must rely on daily food intake to meet the energetic demands of reproduction (Drent & Daan 1980). It has recently been suggested, however, that any micronutrient with the potential to be stored could produce carry-over effects (Harrison *et al.* 2011). In particular, large amounts of fat-soluble dietary antioxidants such as vitamin E and carotenoids can accumulate in subcutaneous fat and liver in birds, forming reserves which may be drawn upon during the breeding season when demand is increased (Negro *et al.* 2001; Surai 2007; Metzger & Bairlein 2011). Antioxidants can have important effects on life histories. For example, vitamin E and carotenoids provide antioxidant defence against reactive oxygen species (ROS) which result as by-products of metabolism (reviewed by Catoni, Peters & Schaefer 2008; Monaghan, Metcalfe & Torres 2009). In doing so, they prevent important biomolecules from being damaged by ROS (i.e. oxidative stress), which might otherwise lead to physiological dysfunction, disease progression and aging. However, we are not aware of any study which has tested whether winter provisioning with an antioxidant may have carry-over effects on reproduction in any species.

Whilst it is generally assumed that provisioning of food to wild birds is beneficial at the individual- and population levels, in fact this is far from clear (Robb *et al.* 2008a; Harrison *et al.* 2010). For example, although winter provisioning may enhance short-term survival, it could result in dependency on feeders, reduced dietary diversity and could even have direct deleterious effects on health (Jones & Reynolds 2008). For example, studies of laboratory rodents have shown that increased dietary carbohydrate and fatty acid availability up-regulates mitochondrial respiration (Iossa *et al.* 2002), which can result in increased susceptibility to oxidative stress (e.g. Igosheva *et al.* 2010). Therefore, it is conceivable that winter provisioning of birds could have negative downstream effects on reproductive capacity, but this remains to be experimentally tested. The potential for such deleterious effects seems likely to depend on the nutritional composition of foods. For example, winter provisioning with macronutrients such as fats could in theory impair subsequent reproduction, whereas dietary antioxidants may mitigate any such deleterious effects of macronutrient metabolism.

One aspect of reproduction which seems especially likely to be influenced by winter provisioning is egg production. In birds, egg production carries high costs both in terms of energy and nutrient requirements (Perrins 1996). The egg's lipid- and protein-rich yolk provides the main source of energy for developing embryos and variation in yolk mass can influence offspring survival (Williams 1994). Nevertheless, it appears that energy supply *per se* is rarely limiting for egg production (e.g. Bolton, Houston & Monaghan 1992). The yolk also contains maternally-derived antioxidants including carotenoids and vitamin E, deposition of which is thought to comprise a maternal effect to enhance offspring performance (McGraw, Adkins-Regan & Parker 2005). The availability of diet-derived antioxidants is

potentially limiting for egg production due to scarcity of antioxidants in the environment and/or physiological trade-offs in their usage (Blount, Houston & Møller 2000). Increased deposition of carotenoids into yolk, resulting from supplemental feeding just prior to and during laying, has been shown to result in reduced yolk susceptibility to lipid peroxidation (Blount *et al.* 2002a; McGraw 2005), and enhanced hatching success (Møller, Karadas & Mousseau 2008), immunity (Saino *et al.* 2003), nestling plumage colouration (Biard, Surai & Møller 2005), and adult survival and sexual signal expression (McGraw, Adkins-Regan & Parker 2005). There is also considerable evidence that yolk-derived vitamin E can function as a potent antioxidant and immunostimulant *in vivo* (reviewed by Surai 2007). However, few studies have investigated whether limitations in the supply of vitamin E may underlie life history trade-offs (but see de Ayala, Martinelli & Saino 2006). Remarkably, there have been no studies of the effects of vitamin E availability on egg production in wild birds, and whether a female's capacity to produce high quality eggs is affected by her access to dietary antioxidants in the preceding winter awaits study.

Any effects of winter provisioning seem likely to be context-dependent, being affected by habitat quality and natural food availability (Kallander 1981). Therefore it is important to study the ecological consequences of dietary provisioning at a landscape scale, across multiple sites and years (Robb et al. 2008a). Here, we use such an approach to examine the effects of winter provisioning of wild blue tits on egg production during the following spring. To investigate the roles of energy and antioxidants as possible mediators of carry-over effects, populations were fed either fat, fat-plus-vitamin E or remained unfed (controls). Our aim was to determine whether winter provisioning altered the timing of laying, clutch size, or egg quality in terms of the relative mass of egg components, and levels of yolk antioxidants (vitamin E and carotenoids). We hypothesised that compared to unfed controls, winter

provisioning with fat would have no beneficial effects on egg production, whereas winter provisioning with fat-plus-vitamin E would enhance egg production. Alternatively, if winter provisioning with a macronutrient such as fat impairs future egg production compared to unfed controls, we hypothesised that this impairment would not be seen in birds provisioned with fat-plus-vitamin E.

## MATERIALS AND METHODS

### Study site and experimental design

The winter provisioning experiment was conducted over three years from 2007 to 2009 and carry-over effects were measured during the subsequent breeding seasons, 2008 – 2010 respectively. The study took place in Cornwall, UK, at nine deciduous woodland sites, where oak (*Quercus* spp.), beech (*Fagus sylvatica*), sweet chestnut (*Castanea sativa*) and sycamore (*Acer pseudoplatanus*) were the predominant tree species. Sites averaged 10.7 hectares in size and were situated at least 2km apart to minimise the possibility of between-site movements of birds. There was no evidence from adult ringing records that birds moved sites between winter and spring, or from year to year. Sites were nominally grouped into three triplets, according to similarities in the composition of tree, understory and ground cover species. Woodland size, proximity to settlements, level of public access and amount of periphery woodland were also taken into account.

In the first year, each site within a triplet was randomly allocated to one of three provisioning groups: (1) no supplement (hereafter 'control'), (2) fat only (hereafter 'fat') and (3) fat-plus-vitamin E (hereafter 'fat+VE'). Treatments were rotated within triplets across years, so that every site received all three treatments over the course of the study. Since treatments were

188 replicated three times using different triplet groups in a given year, any potential confounding
189 effects of year have been avoided.
190
191 Within the six fed sites each year, feeders were hung *ca*. 4m from the ground at 100m
192 intervals along parallel transects (100m apart) and at an average of nine per site. Feeders were
193 custom-designed to prevent access by grey squirrels (*Sciurus carolinensis*) and other
194 mammals and larger bird species. A total of 346 nest boxes, with a 32mm diameter entrance
195 hole, were positioned across all sites (mean ± SE per site: 38 ± 1.4). Boxes were erected at
196 25m intervals along transects and parallel to feeders, such that each box was no more than
197 50m from a feeder. This design produced an equal density and distribution of feeders and
198 boxes across individual woodlands, *ca*. one feeder and four boxes per hectare.

199 **Winter provisioning experiment**

200 Food was provisioned through the winter only (14 Dec – 4 Mar 2007/08; 18 Nov – 11 Mar
201 2008/09 and 2009/10), leaving a gap of at least one month before laying commenced (8 Apr,
202 11 Apr, 15 Apr respectively) and thus allowing carry-over effects to be investigated with
203 confidence. All feeding stations were provisioned with a fresh 150g fat ball every 10 days.
204 Fat balls for the fat+VE treatment group were supplemented with α-tocopherol (T3251;
205 Sigma-Aldrich, Dorset, UK) at a concentration of 100 mg $kg^{-1}$fat, a level equivalent to that
206 found in peanuts (Chun, Lee & Eitenmiller 2005), a popular food provisioned to garden birds.
207 α-Tocopherol cannot be provisioned to wild birds without the use of a 'carrier' and, as a
208 lipophilic molecule, it is inevitably co-acquired with fat in natural foods (Blount *et al.* 2002b).
209 Therefore, the fat+VE treatment group provides an ecologically realistic test of the effects of
210 antioxidant provisioning.
211

All fat balls were produced from solid vegetable fat (Crisp 'n Dry, Princes Ltd., Liverpool, UK) 1 – 2 days in advance of provisioning, using standardised methods adapted from Blount et al. (2002b). Fat was heated to 60$^{o}$C for *ca*. 1 hour until liquefied, then cooled on ice until viscous. When the fat reached 18 – 20$^{o}$C, yellow food colouring (0.125ml/ 100g fat; ASDA Natural Food Colouring, Asda Stores Ltd., Leeds) was added, to increase fat ball attractiveness to target species (McGraw *et al.* 2006) verified by a pilot study (unpubl. data). At the same time α-Tocopherol was added to fat (fat+VE treatment) at the concentration specified above and stirred thoroughly for 4 mins to homogenise. Fat balls (150g) were hardened at -20$^{o}$C overnight before being deployed. Upon collection fat balls were weighed (±0.01g) to determine levels of consumption. Observations at feeders, beak markings on fat balls and winter mist netting confirmed food use was dominated by Parid sp., with a mean of 3.32 kg (± 0.12 SE) consumed per site per year. Ring recoveries and stable isotope analysis provided evidence that winter provisioned foods had been utilised by breeding birds (Plummer 2011). Food uptake per 10 day feeding period differed between years, but was not significantly different between treatment groups (general linear mixed model [GLMM] with site/ feeder random factor; treatment: $\chi_1^2$ = 0.36, $p$= 0.55, year: $\chi_2^2$ = 336.29, $p$< 0.001, treatment × year: $\chi_2^2$ = 0.03, $p$= 0.98).

**Breeding parameters**

Nest boxes were inspected every 1 – 3 days from April to June. Lay date of the first egg was back-calculated by assuming one egg was laid per day, if more than one egg was present (Perrins 1996). After the first egg was laid, nests were visited every 1 – 2 days until clutch completion and new eggs were marked to establish laying order. Total clutch size was recorded upon clutch completion and total clutch mass determined (±0.1g) using an electronic balance. One egg, typically the last- or second-to-last egg laid, was then removed for

measurement of mass and biochemical analysis. Collected eggs were returned to the laboratory, weighed (±0.001g) and dissected on the day of collection. The yolk was rolled over damp filter paper to remove traces of albumen, weighed (±0.001g) and stored at -80$^{o}$C until analysis.

**Biochemical assays**

For extraction of antioxidants, egg yolk (0.040-0.050g) was vortexed in 0.7mL 5% NaCl for 5 seconds and then homogenised with 1mL EtOH for 20 sec. Hexane (1.5mL) was added and samples were further homogenised for 10 sec, before being centrifuged for 4 minutes at 8000 × g and the hexane phase containing the antioxidants drawn off. Extraction was repeated and both hexane extracts combined.

Total carotenoid concentrations in egg yolk were determined by spectrophotometry at 450nm (Nicolet Evolution 500; Thermo Electron Corp., Hemel Hemstead, U.K.) with total carotenoid concentration calculated using the extinction coefficient of lutein in hexane (2589, Craft & Soares 1992). Hexane (500µl) was evaporated to dryness and the residue re-dissolved in 150µl DCM and 150µl MeOH. For determination of α-tocopherol concentrations, samples (20µl) were injected into a high-performance liquid chromatography system (HPLC; Dionex Corporation, California, USA). Separation utilised a 3µ $C_{18}$ reverse-phase column (15 cm x 4.6 mm) (Spherisorb S30DS2; Phase separations, Clwyd, UK), with a mobile phase of MeOH:water (97:3 v/v) at a flow rate of 1.1mL min$^{-1}$. Fluorescence detection (Dionex RF2000) was performed at 295nm (excitation) and 330nm (emission). The α-tocopherol peak was identified and quantified by comparison with a standard solution of α-tocopherol (T3251 Sigma-Altrich) in methanol. Total carotenoid and α-tocopherol concentrations are reported as µg g$^{-1}$ yolk.

**Statistical analyses**

To test the influence of winter provisioning on egg production, general linear mixed models (GLMM) were applied to the following response variables: lay date; clutch size; clutch and egg mass; and yolk α-tocopherol and total carotenoid concentrations. A $\log_{10}$:$\log_{10}$ GLMM of yolk mass on egg mass was used to examine proportionality of yolk investment. Nest box identity nested within woodland site was specified as the random term, to control for temporal and spatial pseudoreplication. An information-theoretic approach based on Akaike's Information Criterion (AIC) was then used for model selection and model averaging (Burnham & Anderson 2002), appropriate for complex large-scale field investigations as reported here (Whittingham *et al.* 2006).

All first clutches (n= 467) were included in lay date analysis, whilst clutch and egg component analyses excluded clutches with laying breaks >2 days (n= 23) and/or abandoned before incubation (n= 32). Eggs showing any sign of incubation upon dissection (i.e. visible early-stage embryo) were excluded from egg component analyses (16% of eggs collected). For each analysis, a candidate set including all possible models given the predictor variables (Table 1), plus a null model fitted with only the intercept, were compared. A quadratic function of lay date (lay date squared) was initially included in to test for non-linear relationships, but it did not improve model fit according to AICc and had little predictive power (main effect parameter estimate ($\beta$) < 0.0001 in all cases) and was therefore excluded from further analyses. Normality and homoscedasticity of residuals were checked prior to model selection; concentrations of α-tocopherol and total carotenoids were subsequently log-transformed to correct normality.

Models were compared using AICc (i.e. AIC corrected for small sample size), where the best fitting model has the lowest AICc value and all other models are ranked according to their difference in AICc from the top model (ΔAICc). If a single 'best' model could not be identified, model averaging was applied across the most strongly supported models (the confidence set), defined by ΔAICc ≤ 2.0 (Burnham & Anderson 2002). Akaike weights ($w_i$) were used to assess the relative support of models within a confidence set, calculate model-averaged parameter estimates ($\beta$) and associated standard errors (SE) and estimate relative importance of explanatory variables ($w$). The predictive power of top ranking models was assessed by calculating a pseudo-$R^2$ value following Nagelkerke (1991), since coefficient of determination ($R^2$) cannot be generated directly for mixed models. Where treatment (or a specific treatment interaction) was well supported for inclusion in the best model, further testing was applied to assess between treatment group differences. Using AICc, the top GLMM model within the confidence set was compared to replicate models in which two focal treatment groups were paired. In this instance, strong support for a between treatment group difference was concluded if the model in which the two groups were paired was ΔAICc > 2 from the original GLMM model. All statistical analyses were conducted in R version 2.12.2 (R Development Core Team 2011) using libraries nlme (Pinheiro *et al.* 2010) and MuMIn (Bartoń 2011).

## RESULTS

### Timing of laying

Lay dates did not differ between treatment groups, but were strongly predicted by between-year differences (mean ±SE per year: 26.6 ±0.6; 24.7 ±0.5; 30.2 ±0.5 respectively, where 1= 1 April). The top model, featuring year only ($w_i$= 0.993, n= 467, pseudo-$R^2$= 0.109), was at

least 141 times better supported by the data than all alternatives within the candidate set which also included treatment and the treatment × year interaction ($w_i$> 0.007).

**Clutch size and relative mass of egg components**

There was strong support for an effect of treatment on the relationship between egg mass and yolk mass; all models within the confidence set for proportionality of yolk investment included the treatment × $\log_{10}$ (egg mass) interaction (Table 2, n= 299, Fig. 1). *Post-hoc* testing revealed that this was driven by differences between the fat and fat+VE treatment groups (ΔAICc= 8.836, for paired model against top model). Whilst fat-fed females had proportionally small yolks compared to controls, fat+VE females produced proportionally larger yolks as egg mass increased (Table 3). But there was no evidence that proportional yolk investment differed between the control group and the fat (ΔAICc= 1.672) or the fat+VE treatment groups (ΔAICc= 0.413). The importance of year, clutch size and lay date in the model was relatively smaller (Table 3).

Winter provisioning treatment explained less than half the variation in clutch size compared to year or lay date when excluding possible interactions ($w$= 0.435; Table 2, models 1 and 2 only, n= 413) and was poorly estimated as indicated by relatively high standard errors (Table 3).

Variation in total resource deposition was investigated in an analysis of clutch mass, controlling for clutch size. Clutch mass was not influenced by treatment and similarly neither was individual egg mass (Table 2, n= 388 and 312 respectively; no models contained treatment or treatment-interactions within 2 ΔAICc confidence sets). Year was the best predictor of total clutch and individual egg mass variation; females laid clutches of reduced

mass in 2009 and had comparatively large eggs in 2008 ($w$= 1.000; Table 3). However the models within the egg mass confidence set explained only a small proportion of the variation (pseudo-$R^2$= 0.046 and 0.047; Table 2). Furthermore, a variance components analysis of model 1 (Table 2), using restricted maximum likelihood (REML), revealed that 71.7% of egg mass variation was attributed to inter-clutch variation, with woodland site accounting for 3.5% and nest box for 24.9%, indicating that inherent differences among females were the greatest predictor of egg mass variation.

**Egg yolk antioxidants**

Variation in yolk α-tocopherol concentration was not affected by winter provisioning treatment. In this analysis the best supported model received a high level of support compared to all others in the candidate set (unadjusted $w_i$= 0.748; Table 2), suggesting that differences were the result of annual and seasonal variation. α-Tocopherol levels were lower in 2009 and decreased as the season progressed, but to a greater extent in larger clutches (Table 3).

By contrast, evidence of a difference in total carotenoid concentration between the treatment groups received a high level of support in an interaction with lay date (Table 2). *Post-hoc* comparisons revealed that yolk total carotenoid concentration in relation to laying date differed between the fat-fed treatment group compared to fat+VE (ΔAICc= 12.346, for paired model against top model) and control groups (ΔAICc= 2.469), whilst the fat+VE and control groups were comparatively similar (ΔAICc= 1.580) (Table 3, Fig. 2). Compared to females of the other groups, fat-fed females invested fewer carotenoids into their eggs early in the season, but more carotenoids later in the season. Both year and the lay date by clutch size interaction also received strong selection probabilities, whereby total carotenoid levels were

reduced in 2010 and showed a seasonal increase, to a greater extent in smaller clutches ($w \geq$ 0.540, Table 3).

## DISCUSSION

The results of this study demonstrate pervasive, downstream effects of winter provisioning on egg production the following spring. However, the effects were strongly influenced by the types of nutrients provided. Previous studies of carry-over effects in birds have considered that macronutrients such as fats are key limiting resources; an increase in dietary macronutrient supply may either result in storage in body tissues for later use, or may result in boosted body condition in one season or year, such that individuals perform better in a subsequent season or year (reviewed by Harrison *et al.* 2011). Our results are striking because they demonstrate that: 1) increased dietary fat availability in winter can in fact impair subsequent egg production in terms of relative yolk mass, and egg carotenoid deposition early in the breeding season; and 2) macronutrients such as fats are clearly not the only nutritional currency that can invoke carry-over effects; negative effects of winter provisioning were not seen in birds fed fat together with vitamin E.

It is well established that larger eggs confer benefits to offspring in terms of growth and survival, with these effects attributed to yolk resources (Williams 1994). But furthermore, as well as providing the major energetic requirements for early development, the yolk comprises a cocktail of micronutrients and maternally-derived compounds known to influence offspring fitness, such as antioxidants, immunoglobulins and hormones (Gasparini *et al.* 2001; Blount *et al.* 2002b; Groothuis *et al.* 2005). Thus, increasing yolk mass can benefit offspring in terms of enhanced embryonic growth and post-hatching reserves (Peach & Thomas 1986; Bourgault *et al.* 2007). Egg size is a relatively inflexible trait within females, compared to

between-individual differences (Christians 2002). Consistent with this we found no effects of winter provisioning treatment on egg mass *per se*. However, variation in the yolk component as a function of total egg mass reflects the absolute difference in the nutrient and energy content of an egg. As such relative yolk mass is an important determinant of egg quality (Williams 1994), which females may modulate adaptively or due to constraint, depending on their physiological condition and access to resources. Typically in altricial and precocial bird species, yolk mass varies in direct proportion to egg mass (i.e. an isometric relationship; Williams 1994). For a small proportion of females constrained to lay small eggs, fat provisioning appears to have been beneficial. But, as egg mass increased fat-provisioning led to a significant decline in relative yolk mass (negative allometry; Fig. 1).However this deleterious consequence of winter provisioning was not seen in birds that had received fat together with vitamin E.

How could winter provisioning with fat apparently reduce the capacity of females to produce large egg yolks? As income breeders, blue tits are incapable of storing sufficient amounts of endogenous macronutrients to fuel reproduction (Drent & Daan 1980), therefore fat provisioned in winter is unlikely to have been utilised directly for egg formation several weeks or months later. Instead, yolk mass is a function of food availability in the days leading up to laying in income breeders (Ardia, Wasson & Winkler 2006). It therefore appears that winter provisioning with fat subsequently impaired the capacity of birds to acquire, assimilate and/or mobilise key resources required for yolk formation during egg production. Yet, the addition of vitamin E to fat supplements mitigated this. We think the most likely explanation is that birds may come to rely on readily accessible provisioned foods in winter (Brittingham & Temple 1992). Whilst a high-fat diet provides an abundance of energy, it could mean that birds fail to obtain a balanced, natural diet and are lacking in key

404 nutrients such as antioxidants. Indeed, a high fat diet should increase the requirement for
405 antioxidant protection, polyunsaturated fatty acids in particular being highly susceptible to
406 oxidative damage (Igosheva *et al.* 2010). In our study it was logistically impossible to follow
407 individual birds throughout winter and spring, and thus we were unable to assess effects of
408 provisioning on oxidative stress levels during winter and to relate this to breeding
409 performance. However, it seems possible that fat-fed females may have suffered oxidative
410 stress which impaired their ability to invest resources in egg composition. Vitamin E is a
411 potent antioxidant, capable of breaking the chain of lipid peroxidation (Surai 2007), and
412 therefore acquisition of dietary vitamin E could have mitigated the oxidative burden imposed
413 by a fatty diet.
414
415 Yolk α-tocopherol concentrations reduced over the laying period and were unaffected by
416 winter provisioning treatment. It is therefore unlikely that α-tocopherol acquired through
417 winter provisioning was stored for later use during egg production. We found no significant
418 effect of provisioning treatment on laying date. However, compared to both unfed and
419 fat+VE females, individuals provisioned with fat alone produced eggs with relatively low
420 concentrations of carotenoids early in the season, but relatively high concentrations of
421 carotenoids later in the season. There is strong selection for breeding early in blue tits, as in
422 many other bird species, because early breeders afford their offspring higher survival and
423 recruitment prospects (Perrins 1996). It has been shown that supplementing the diet of blue
424 tits with carotenoids just prior to and during laying results in significantly elevated levels of
425 carotenoids in eggs (Biard, Surai & Møller 2005). Increased levels of yolk carotenoids can
426 counter lipid peroxidation (Blount *et al.* 2002a; McGraw, Adkins-Regan & Parker 2005), to
427 which the lipid-rich yolk and rapidly growing embryo are highly susceptible, and have a
428 range of beneficial effects in nestlings such as increased immunity and survival (Saino *et al.*
429 2003; Biard, Surai & Møller 2005; McGraw, Adkins-Regan & Parker 2005). However,

females in poor condition or experiencing oxidative stress may deposit fewer carotenoids into their eggs in favour of somatic maintenance (e.g. Hõrak, Surai & Møller 2002; Blount *et al.* 2004; Isaksson, Johansson & Andersson 2008). It is perhaps more important to lay early than to produce eggs that contain high levels of carotenoids. It seems likely that in our study, fat-fed birds attempted to lay at the optimal time even though it was at the cost of producing eggs that contained relatively low levels of carotenoids. Potentially, this could be amplified if winter provisioning enabled relatively low-quality individuals to enter the breeding population, which would otherwise not have bred at all. In the fat-fed treatment group, where nutrient acquisition appeared to have deleterious consequences for egg production, a change in phenotypic structuring could have resulted in reduced average egg quality at the population level.

It is possible that the influence of winter provisioning reduced as the season progressed, due to the increase in time between food uptake and egg laying. However, we do not know why fat-fed birds that laid late produced eggs with relatively high concentrations of carotenoids, compared to females of the other provisioning treatments. It is well established that dietary access to carotenoids increases over the laying season in parids. Caterpillar supply, which provides the main food resource for breeding tits, increases in number and quality across the laying period (Arnold *et al.* 2010). The increase in carotenoid allocation to eggs later in the season in fat-fed birds was not simply because they produced smaller clutches; although clutch size decreased over the laying season, this decline did not differ significantly amongst provisioning treatments. One possible explanation is that fat-fed birds had low survival prospects, i.e. no expectation of future reproduction, and therefore invested more in current reproduction as the season neared its end (Royle, Surai & Hartley 2003; Alonso-Alvarez *et al.*

2004). We do not have the data to assess this possibility, but this would be an interesting direction for further work.

As urban land cover expands, gardens are expected to play an increasingly important role in the conservation of biodiversity (Chamberlain, Cannon & Toms 2004). The provision of food for garden birds has been thought likely to benefit this objective (e.g. Toms & Sterry 2008), although there is limited and indeterminate evidence of its ecological impacts (e.g. Robb *et al.* 2008b; Harrison *et al.* 2010). More generally, food provisioning has also been applied as a conservation tool to manage endangered populations (e.g. Armstrong, Castro & Griffiths 2007; Oro *et al.* 2008). Our study is the first to report deleterious effects of provisioning which were carried over from one season to the next. We emphasise, however, that our study focussed on egg phenotypes; it will be important to see how these effects translate into fitness consequences. The mechanism by which these negative effects were generated is of key importance; the provision of energy-rich fat supplements in winter had negative consequences for female egg investment several weeks after provisioning stopped. Yet at the population level this was mitigated by the provision of fat together with vitamin E. This is the first direct evidence that the specific nutritional composition of provisioned foods may determine whether carry-over effects on breeding performance are positive or negative at the population level. Therefore, where provisioning is practiced as a conservation tool, careful consideration should be given to the nutritional composition of foods. Whether winter provisioning of garden bird species is considered to be beneficial or deleterious may depend on whether effects are interpreted at the level of individuals, or populations. Provisioning may lead to a reduction in average levels of egg quality at the population level. However, if provisioning enables certain low-quality individuals to breed, when they might otherwise have died or survived only as non-breeders, this would clearly enhance their lifetime

reproductive success and may in fact boost the overall population size. It is evident that further work at the level of individuals is needed to understand how winter feeding may be used to benefit wild bird populations in the future.

## ACKNOWLEDGEMENTS

We thank the many landowners and fieldworkers who made this research possible, Chris Mitchell for assistance in the lab, and Nick Carter for logistical support. Egg collection for biochemical analysis was licensed by Natural England (licence numbers: 20081292, 20091379 and 20100947). This work was funded by a Natural Environment Research Council (NERC) CASE studentship (to KEP, JDB, SB and DEC), a Royal Society Research Fellowship (to JDB), the BTO and Gardman Ltd.

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661 **Table 1.** List of parameters included as fixed explanatory variables in GLMMs to investigate
662 causes of egg production variation. Egg production response variables examined were lay
663 date (LD); clutch size (CS); clutch mass (CM); egg mass (EM); and yolk α-tocopherol
664 (YTOC) and total carotenoid (YCAR) concentrations

| Fixed predictors | Description/levels | Reason for inclusion | Response |
|---|---|---|---|
| Treatment | 3 level factor – unfed, fat, fat+VE | Examine the effect of winter provisioning | LD; CS; CM; EM; YTOC; YCAR; YM |
| Year | 3 level factor – 2008, 2009, 2010 | Life-history traits vary annually (Svensson & Nilsson 1995; Lambrechts *et al.* 2004) | LD; CS; CM; EM; YTOC; YCAR; YM |
| Lay date | Continuous, 1 = 1 April | Clutch size and breeding performance vary seasonally (Norris 1993; Perrins 1996) | CS; CM; EM; YTOC; YCAR; YM |
| Clutch size | Continuous | Account for variation in female breeding condition and trade-offs between egg number and quality (Slagsvold & Lifjeld 1990; Perrins 1996) | CM; EM; YTOC; YCAR; |
| $Log_{10}$ (Egg mass (g)) | Continuous | Examine proportionality of yolk investment | YM |
| Treatment × Year | 2-way interaction | Examine whether effects of annual variation were consistent between treatments | LD; CS; CM; EM; YTOC; YCAR; |
| Treatment × Lay date | 2-way interaction | Examine whether effects of seasonal variation were consistent between treatments | CS; CM; EM; YTOC; YCAR; |
| Treatment × Clutch size | 2-way interaction | Examine whether effects of clutch size were consistent between treatments | CM; EM; YTOC; YCAR; |
| Lay date × Clutch size | 2-way interaction | Improve model fit by controlling for decline in clutch size through laying period (Perrins 1996) | CM; EM; YTOC; YCAR; |
| Treatment × $Log_{10}$ (Egg mass) | 2-way interaction | Examine whether proportional yolk investment was consistent between treatments | YM |

665

**Table 2.** Confidence sets of ranked models for analyses of maternal investment in egg production, based on Akaike's information criterion corrected for small sample size (AICc)

| Rank | Model parameters* | Log-likelihood | AICc | ΔAICc | $w_i$ † | Pseudo-$R^2$ |
|---|---|---|---|---|---|---|
| *(a) Proportional yolk mass ($log_{10}$(yolk mass))* | | | | | | |
| 1 | (treat × $log_{10}$(EM)) | 581.7 | -1144.8 | 0.000 | 0.228 | 0.486 |
| 2 | (treat × $log_{10}$(EM)) + year | 583.8 | -1144.7 | 0.027 | 0.225 | 0.494 |
| 3 | (treat × $log_{10}$(EM)) + LD | 582.4 | -1144.1 | 0.636 | 0.166 | 0.489 |
| 4 | (treat × $log_{10}$(EM)) + CS | 584.3 | -1143.8 | 0.993 | 0.139 | 0.489 |
| 5 | (treat × $log_{10}$(EM)) + year + CS | 584.3 | -1143.6 | 1.215 | 0.124 | 0.496 |
| 6 | (treat × $log_{10}$(EM)) + year + LD | 584.3 | -1143.4 | 1.344 | 0.117 | 0.496 |
| *(b) Clutch size* | | | | | | |
| 1 | year + LD | -756.8 | 1527.9 | 0.000 | 0.444 | 0.142 |
| 2 | year + LD + treat | -755.0 | 1528.4 | 0.530 | 0.340 | 0.149 |
| 3 | year + (treat × LD) | -753.3 | 1529.3 | 1.443 | 0.216 | 0.156 |
| *(c) Clutch mass* | | | | | | |
| 1 | year + LD + CS | -439.14 | 894.7 | 0.000 | 0.717 | 0.857 |
| 2 | year + (LD × CS) | -439.02 | 896.5 | 1.857 | 0.283 | 0.857 |
| *(d) Egg mass* | | | | | | |
| 1 | year + CS | 294.3 | -574.2 | 0.000 | 0.705 | 0.046 |
| 2 | year + CS + LD | 294.5 | -572.5 | 1.746 | 0.295 | 0.047 |
| *(e) α-Tocopherol concentration* | | | | | | |
| 1 | year + (LD × CS) | -210.61 | 439.8 | 0.000 | 1.000 | 0.111 |
| *(f) Total carotenoid concentration* | | | | | | |
| 1 | year + (LD × CS) + (treat × LD) | -79.00 | 185.2 | 0.000 | 1.000 | 0.252 |

* Treat, winter feeding treatment; EM, egg mass; LD, lay date; CS, clutch size; ×, interaction term
† Akaike weight for the model within the confidence set

**Table 3.** Relative variable importance (*w*), model-averaged parameter estimates (Est.) and standard errors (SE) for variables represented in the confidence sets of maternal egg investment analyses

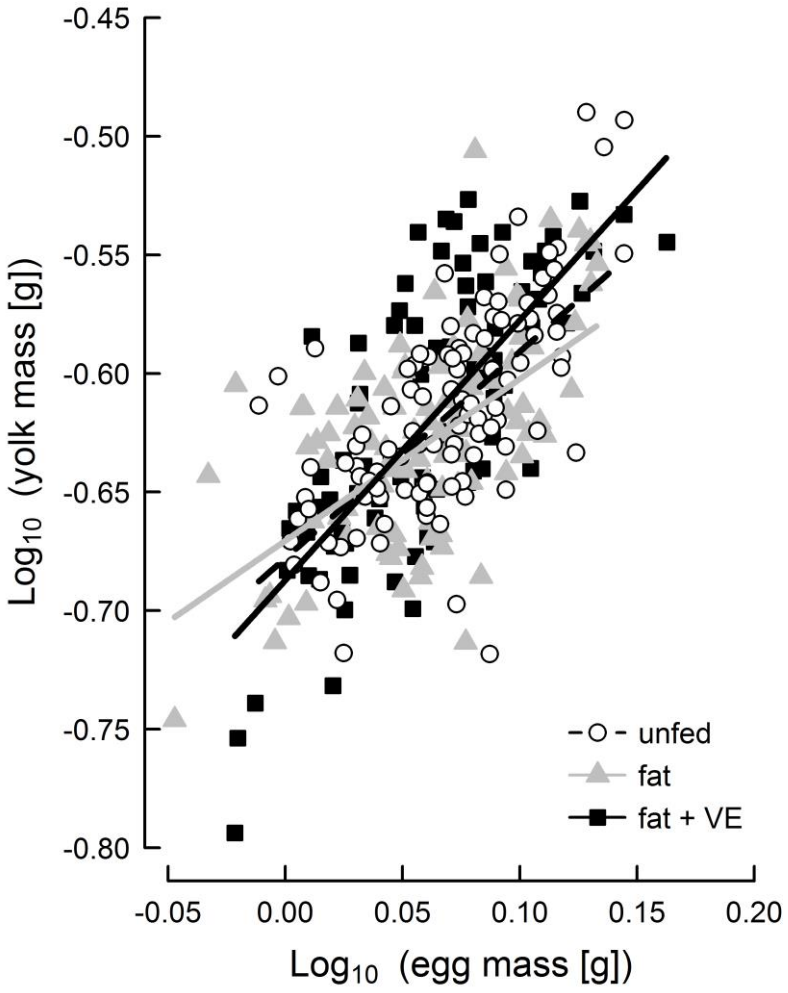
| | Proportion yolk | | | Clutch size | | | Clutch mass | | | Egg mass | | | α-Tocopherol concentration | | | Total carotenoid concentration | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Parameter | *w* | Est. | SE | *w* | Est. | SE | *w* | Est. | SE | *w* | Est. | SE | *w* | Est. | SE | *w* | Est. | SE |
| Intercept | | -0.678 | 0.014 | | 10.931 | 0.487 | | 1.407 | 0.491 | | 1.253 | 0.037 | | 4.189 | 0.508 | | 2.635 | 0.364 |
| Clutch size | 0.263 | 0.000 | 0.001 | | | | 1.000 | 1.035 | 0.054 | 1.000 | -0.008 | 0.003 | 1.000 | 0.125 | 0.057 | 1.000 | 0.110 | 0.039 |
| Lay date | 0.283 | 0.000 | 0.000 | 1.000 | -0.096 | 0.015 | 1.000 | -0.020 | 0.015 | 0.295 | 0.000 | 0.001 | 1.000 | 0.035 | 0.017 | 1.000 | 0.037 | 0.012 |
| $Log_{10}$(egg mass) | 1.000 | 0.870 | 0.102 | | | | | | | | | | | | | | | |
| Treatment | 1.000 | | | 0.556 | | | | | | | | | | | | 1.000 | | |
| fat | | 0.007 | 0.010 | | -0.369 | 0.681 | | | | | | | | | | | -0.315 | 0.176 |
| fat+VE | | -0.009 | 0.010 | | -0.255 | 0.424 | | | | | | | | | | | 0.203 | 0.167 |
| Year | 0.466 | | | 1.000 | | | 1.000 | | | 1.000 | | | 1.000 | | | 1.000 | | |
| 2009 | | 0.004 | 0.006 | | -0.332 | 0.182 | | -0.289 | 0.094 | | -0.033 | 0.013 | | -0.134 | 0.066 | | -0.002 | 0.042 |
| 2010 | | 0.004 | 0.006 | | 0.305 | 0.183 | | -0.069 | 0.094 | | -0.027 | 0.013 | | 0.118 | 0.067 | | -0.159 | 0.043 |
| Lay date × clutch size | | | | | | | 0.283 | 0.000 | 0.002 | | | | 1.000 | -0.006 | 0.002 | 1.000 | -0.004 | 0.001 |
| Treatment × lay date | | | | 0.216 | | | | | | | | | | | | 1.000 | | |
| fat × lay date | | | | | 0.010 | 0.022 | | | | | | | | | | | 0.014 | 0.006 |
| fat+VE × lay date | | | | | 0.002 | 0.012 | | | | | | | | | | | -0.009 | 0.006 |
| Treatment × $log_{10}$(egg mass) | 1.000 | | | | | | | | | | | | | | | | | |
| fat × $log_{10}$(egg mass) | | -0.188 | 0.138 | | | | | | | | | | | | | | | |
| fat+VE × $log_{10}$(egg mass) | | 0.225 | 0.140 | | | | | | | | | | | | | | | |

## LEGEND TO FIGURES

**Figure 1.** Relationship between yolk mass and egg mass. Lines fitted using model averaged parameter estimates. Testing the difference in the slopes (*b*) against 1.0 (isometry) reveals that increases in egg mass are accompanied by a proportionate increase in yolk mass for control ($b$= 0.870 ±0.102(SE); $t_{103}$= 1.23, $p$= 0.200) and fat+VE ($b$= 1.095 ±0.138; $t_{92}$= 0.69, $p$= 0.492) groups. But yolk mass increases proportionately less than egg mass in the fat group (negative allometry; $b$= 0.682 ±0.135; $t_{98}$= 2.35, $p$= 0.021).

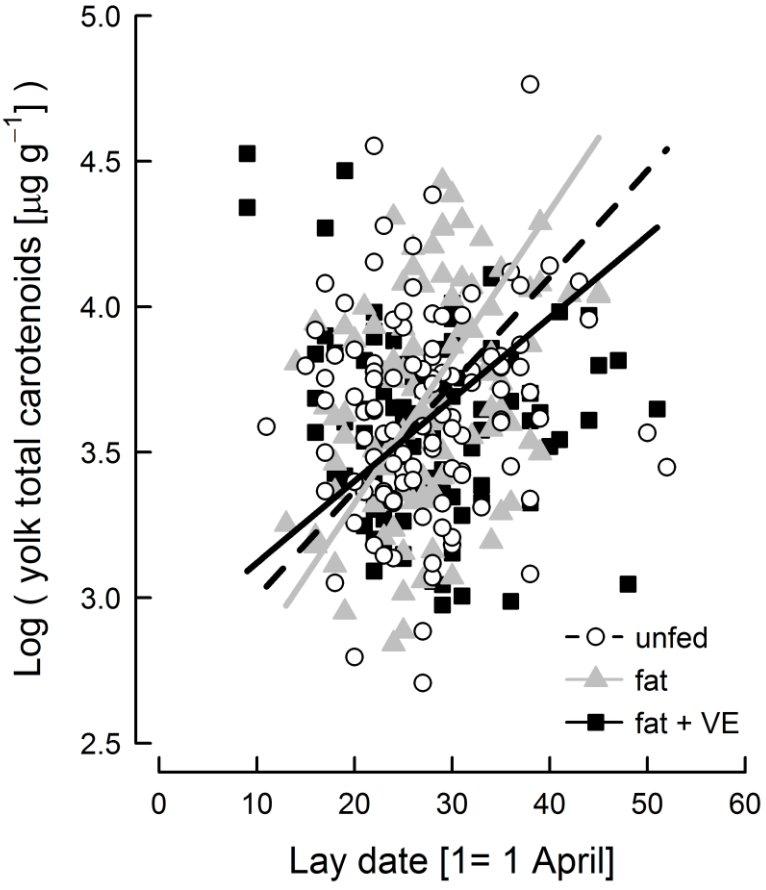
**Figure 2.** Relationship between yolk total carotenoid concentration and lay date. Lines fitted using model averaged parameter estimates. See Tables 1 and 2 for statistical findings and text for details.

684 **Figure 1.**




685

686 **Figure 2.**

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